SOURCE: Biochimica et Biophysica Acta - Lipids and Lipid Metabolism, (1991), 1085/2 (201-208)

5. TITLE: ENZYMATIC MODIFICATION OF LOW-DENSITY-LIPOPROTEIN BY PURIFIED LIPOXYGENASE PLUS PHOSPHOLIPASE-A2
AUTHOR: SPARROW C P (Reprint); PARTHASARATHY S; STEINBERG D
CORPORATE SOURCE: UNIV CALIF SAN DIEGO, LA JOLLA, CA, 92093
COUNTRY OF AUTHOR: USA
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6. TITLE: ENZYMATIC MODIFICATION OF LOW-DENSITY LIPOPROTEIN BY PURIFIED LIPOXYGENASE PLUS PHOSPHOLIPASE-A2 MIMICS CELL-MEDIATED OXIDATIVE MODIFICATION
AUTHOR: SPARROW C P; PARTHASARATHY S; STEINBERG D (Reprint)
CORPORATE SOURCE: UNIV CALIF SAN DIEGO, SCH MED, DEPT MED, DIV ENDOCRINOL & METAB, M-013D, LA JOLLA, CA, 92093
COUNTRY OF AUTHOR: USA
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Basic Science/Circulation: Cellular/Molecular Biology II

Wednesday Afternoon

1903 Relationships Between DNA and Protein Polymorphisms of Apolipoprotein B

Alison M. Dunning, Christian Ehnholm, Rene Butler, Steve E. Humphries, Matti J. Tikkanen, University of Helsinki, Finland.

Genetic variants of Apolipoprotein B (ApoB) may be involved in the determination of serum lipid levels. The association between four Restriction Fragment Length Polymorphisms (RFLPs) of the gene for Apo B and five antigen group (Ag) protein polymorphisms of Apo B have been investigated in 22 unrelated Finnish individuals. In this sample a complete correlation exists between the EcoRI RFLP and the Ag (t/z) polymorphism. There is strong association between the alleles of the XbaI RFLP and Ag (c/g). Furthermore there is an association of the same XbaI polymorphism with Ag(x/y). Linkage disequilibrium was discovered between the PvuII RFLP and the Ag (a_1/d) polymorphism. These associations confirm that the Ag variants are true protein sequence polymorphisms of Apo B.

1904 Epitope Location and Modulation in Apolipoprotein A-I : Lipids and Apolipoprotein A-II Requirement.

F. Pio, N. Vu-Dac, V. Clavey, J-C Fruchart. SERLIA, Institut Pasteur, Lille, France

Monoclonal antibodies A17 and A30 were studied by competitive inhibition with ^{125}I HDL_3 for their expression on high density lipoprotein (HDL) subclasses and on dimyristoyl phosphatidylcholine (DMPC) apolipoprotein A-I and apolipoprotein A-II complexes. Immunoblotting on apo A-I cyanogen bromide (CNBr) fragments located the A17 on CNBr4 (148-243) and A30 on CNBr1 (1-86). A17 antigenic determinant is expressed identically on HDL subclasses, DMPC apo A-I and on DMPC with apo A-I/apo A-II molar ratios 2:1 and 1:2. The A30 antibody recognized apo A-I 5 times better when the protein was associated with HDL_2 and 4 times better when associated with HDL_3. Association with DMPC also restored epitope expression to the same extent. Addition of apo A-II to the complex increased the antigenicity of the A-I 2.5 times when the apo A-I/apo A-II ratio was 2:1 and 4 times when the ratio was 1:2.

We conclude that expression of the A17 epitope is largely independent of the protein conformation while expression of the A30 epitope is conformation dependent and involve lipid protein interaction.

1905 Enzymatic Modification of Low Density Lipoprotein by Purified Lipoxygenase Plus Phospholipase A_2

Carl P. Sparrow, Sampath Parthasarathy, and Daniel Steinberg, University of California, San Diego, La Jolla, CA.

Cultured endothelial cells can oxidize LDL, resulting in increased uptake of the modified lipoprotein by macrophages. Previous studies showed that phospholipase A_2 activity associated with LDL is essential but the source of the active oxygen involved has not been determined. In the present studies we show that incubation of LDL with purified soybean lipoxygenase together with purified phospholipase A_2 can mimic endothelial cell-modification of LDL. Typically, incubation with lipoxygenase plus phospholipase generated about 15 nmoles of thiobarbituric acid-reactive substances per mg of LDL protein; a 4- to 7-fold increase in subsequent macrophage degradation of the LDL; a 10-fold decrease in fibroblast degradation of the LDL; and a marked increase in electrophoretic mobility. Thus all of the changes accompanying cell-induced oxidative modification could be mimicked. The macrophage degradation of LDL treated with lipoxygenase/phospholipase was inhibited by copper-oxidized LDL, fucoidin and polyinosinate, showing that recognition was largely by way of the acetyl-LDL receptor. This is the first example of biological oxidative modification of LDL induced by a purified enzyme system.

1906 Characterization of the Export and Processing of Human Preproapo AI Expressed in Murine AtT-20 Cells

Susan M. Fennevald and Jeffrey I. Gordon, Washington University, St. Louis

The molecular details of hov human HDL apolipoproteins are transported within the secretory apparatus remain ill defined. The export and processing of apo AI vere studied in a murine pituitary cell line (AtT-20) which does not normally synthesize lipoproteins. The human apo AI gene vas cloned into pSV2neo downstream from a metallothionein promoter and transfected into AtT-20 cells. These cells possess 2 export pathways; entry into the regulated pathway can be distinguished from entry into the constitutive pathway by segregation into secretory granules and stimulation of secretion by cAMP analogues. Western blot analysis indicated that apo AI synthesized in AtT-20 cells enters the regulated pathway - it is released in response to 8Br-cAMP treatment and is associated with secretory granules isolated on D_2O-Ficoll gradients. Edman degradation of labeled intracellular and media apo AI revealed that its NH_2-terminal prosegment is not removed prior to or after secretion. Density gradient ultracentrifugation studies of media disclosed that ~15% of the exported proapo AI becomes associated with lipids either during or after export. This model system may be useful for defining structural domains in apo AI which regulate its intracellular transport, compartmentalization and ligand interaction.